The Origin and Development of the Spermatophoric Mass of a Nephropsid Lobster, *Enoplometopus occidentalis* (Randall)[1]

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INTRODUCTION

The purpose of this paper on the origin and development of the spermatophoric mass of *Enoplometopus occidentalis* (Randall) is threefold: (1) to increase our knowledge of the biology of this little-known species, (2) to show evidence in support of external fertilization, and (3) to extend the list of mechanically sperm-liberating families in Hawaii to include the Nephropsidae (Homaridae *auct.*, Nephropsidae *fide* Holthuis, 1946).

With the possible exception of the Cape crayfish (*Jasus lalandii*, von Bonde, 1936), all recorded observations indicate that the palinurids, the astacids, and the homarids produce continuous, highly convoluted spermatophoric tubes embedded in putty-like matrices (*Panulirus interruptus*—Allen, 1916; Fasten, 1917; Wilson, 1949; *P. argus*—Crawford and De Smidt, 1923; *P. penicillatus*—Matthews, 1951; *Potamobius trowbridgii*—Andrews, 1931; *Homarus americanus*—Herrick, 1895).

Although the process has not been observed in every case, female pereiopodal gouging of these spermatophores during oviposition probably allows the liberation of their spermatozoa and the subsequent external fertilization.

Among macruran spermatophores studied, mechanical liberation of sperm reaches its highest development in the scyllarid *Parribacus antarcticus* (Lund). The spermatophoric mass resembles that of the palinurids, the astacids, and the homarids, but certain of the pereiopods are provided with special structures for the mechanical liberation of the spermatozoa (Andrews, 1912; Matthews, 1953). Here, likewise, fertilization is probably external.

Although once obtainable from Honolulu markets (*vide* Rathbun, 1906: 900), *E. occidentalis* is now seldom seen here except at the Honolulu Aquarium, although it is occasionally taken on the reefs and at depths of a few fathoms (*vide* Edmondson, 1946: 257). Yet large numbers of *Enoplometopus* postlarvae found in the stomachs of the yellowfin tuna *Neothunnus macropterus* (Reintjes, unpubl. ms.) attest its prevalence in deeper water. Although only males were obtainable for dissection, a single female in the collection of the Bernice P. Bishop Museum was available for observation. This specimen was devoid of spermatophoric mass. The spermatophoric mass of *E. occidentalis* in so far as can be ascertained has not been investigated.

The writer wishes to thank Mr. Spencer Tinker, director of the Honolulu Aquarium, who furnished the living specimens.

METHODS AND TECHNIQUES

Mature male specimens of *E. occidentalis*, obtained from the Honolulu Aquarium in November, 1952, were used in this study. The reproductive organs were removed, fixed in either Bouin's or Zenker's fluid, dehydrated and cleared in dioxane, embedded in Tissuemat (54–56°C.), and sectioned at 10 microns. The mounted sections were stained with standard alumhaematoxylin and counterstained with eosin.

Certain vasa efferentia were placed in 0.1 N KOH. Others were dissected, the spermatophoric mass smeared on small glass plates, and these immersed in sea water.

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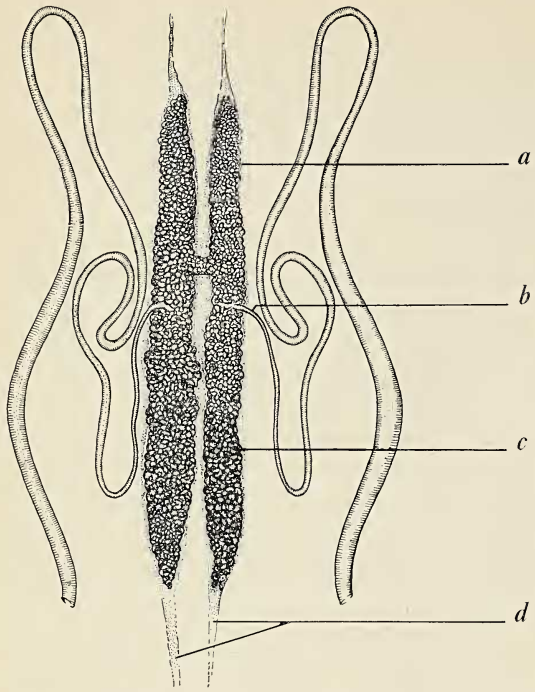

FIG. 1. Male reproductive system of *E. occidentalis* dissected to show: *a*, anterior region of testis; *b*, proximal portion of vas deferens; *c*, posterior region of testis; *d*, mesenteries. (2.5×)

RESULTS

Dissection of *E. occidentalis* reveals an H-shaped testicular mass (Fig. 1), the anterior portion of which (*a*) lies dorsal to the hepatopancreas and the posterior (*c*) contiguous with the digestive tract. The testes are held in place by mesenteries (*d*) and are abundantly supplied with blood vessels.

The slightly coiled vas deferens (Fig. 1*b*), which arises from the testis, increases in diameter distally and opens on the coxopodite of the fifth pereiopod. The entire vas deferens is quiescent and, even when pinched with forceps, fails to eject its spermatophoric mass from the genital pore. There is no evidence of a hyaline line (*vide* Matthews, 1951).

In cross sections, follicles in all stages of maturity are seen throughout the testis. An immature follicle (Fig. 2) discloses large, primary spermatocytes (*c*), indistinguishable from the germinal epithelial cells (*a*) from which they originate. Cells with indistinct cytoplasm but with strongly chromatophilic nuclei (*b*) appear scattered indiscriminately among the primary spermatocytes.

Cross sections of maturing follicles (Fig. 3) reveal that as the number of spermatids (*a*) increases, the number of primary spermatocytes (*b*) is reduced until ultimately a single, peripheral, germinal layer remains. The number of cells with strongly chromatophilic nuclei (*c*) also increases throughout the follicles.

Cross sections of mature follicles (Fig. 4) show the spermatids metamorphosed into spermatozoa (*a*) and an increase in the number of primary spermatocytes (*b*). The longitudinal sections of the collecting tubule (*c*) reveal that spermatozoa (*a*) which emanate from any one follicle remain in groups (*d*), and, although the cells with strongly chromatophilic nuclei (*e*) are numerous in the emptying follicle, they are seldom seen among the groups of spermatozoa in the tubule. A secretion (*f*) from the epithelial lining (*g*) of

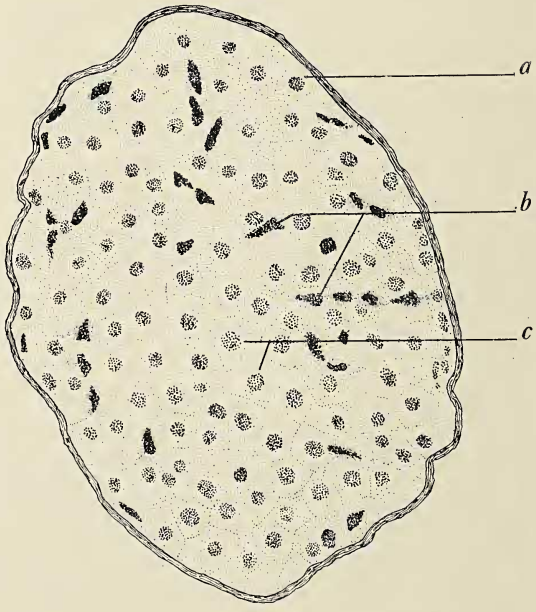

FIG. 2. Camera lucida drawing of a cross section through an immature follicle showing: *a*, germinal epithelium; *b*, nuclei of sustentacular cells; *c*, primary spermatocytes. (175×)

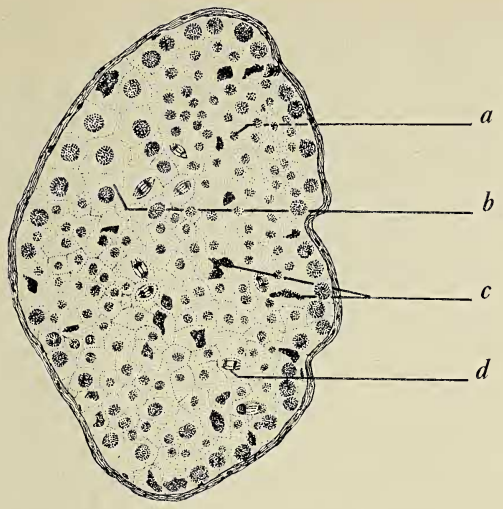

FIG. 3. Camera lucida drawing of a cross section through a follicle in the early stages of maturity showing: *a*, spermatids; *b*, primary spermatocytes; *c*, nuclei of sustentacular cells; *d*, meiotic figures of spermatid formation. (175×)

the tubule surrounds each group of spermatozoa.

Cross sections of the minute, proximal portion of the vas deferens (Fig. 5) reveal a tube in which the sperm mass (*a*) is surrounded by columnar epithelium (*b*) and a muscular layer (*c*). Although the columnar epithelial cells appear active, there is no evidence, in this region of the vas deferens, that a sheath is being formed around the sperm mass.

A portion of a cross section of the vas deferens (Fig. 6) (distad to the portion illustrated in Fig. 5) shows the sperm mass (*a*) enveloped by a spermatophoric sheath (*b*) and that this sheath is the product of the secretion from the epithelial cells (*c*). The thickness of the muscular layer (*d*) is only slightly increased over that illustrated in Figure 5.

Figure 7 illustrates a portion of a cross section through the distal region of the vas deferens. The sperm mass (*a*) is not only enveloped by a spermatophoric sheath (*b*), but a homogeneous matrix (*c*) is added by the epithelial cells (*e*). The matrix (*c*) is often separated from the epithelial cells (*e*) by a space (*d*). The muscular layer (*f*) is increased in thickness and consists of longitudinal, diagonal, and circular fibers.

DISCUSSION

By mitotic division of the germinal epithelial cells (Fig. 2*a*), follicles fill with primary spermatocytes (*c*). During this process, certain cells with strongly chromatophilic nuclei (*b*) apparently arise from the germinal epithelial cells and occupy positions between the primary spermatocytes (*c*). Although mitotic figures are associated with the increase of primary spermatocytes, no mitotic figures accompany the increase of the strongly chromatophilic cells. Their method of multiplication remains obscure.

Concurrently, the primary spermatocytes (Fig. 3*b*) by two successive divisions (*d*) give

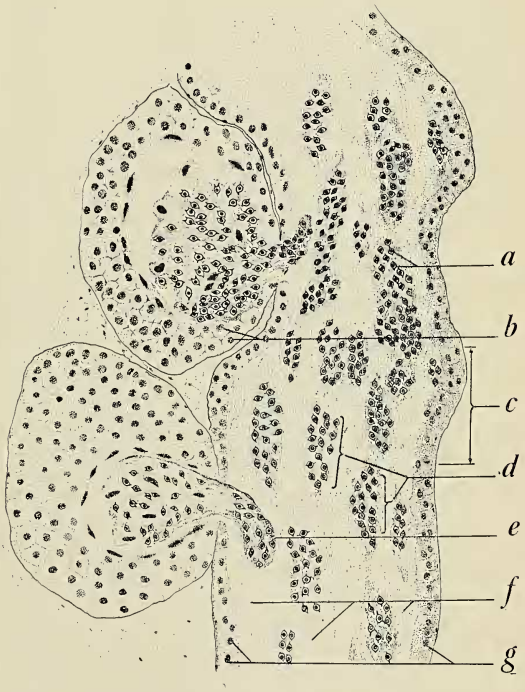

FIG. 4. Camera lucida drawing of cross section through mature follicles discharging their contents into the collecting tubule showing: *a*, spermatozoa; *b*, primary spermatocytes; *c*, longitudinal section of collecting tubule; *d*, groups of spermatozoa ejected from different follicles; *e*, contents of a single follicle being ejected into collecting tubule; *f*, secretion separating sperm groups; *g*, epithelial cells. (150×)

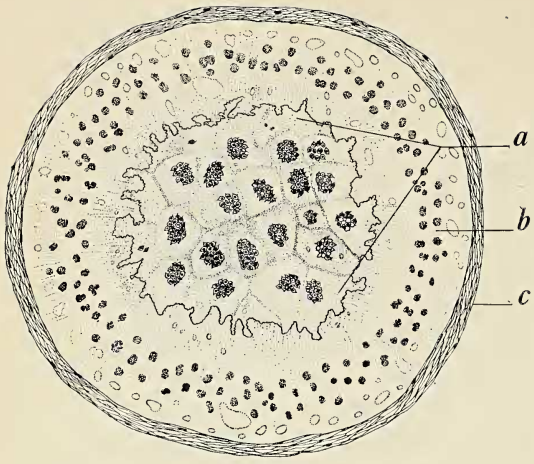

FIG. 5. Camera lucida drawing of a cross section through the minute, proximal portion of the vas deferens showing: *a*, sperm mass; *b*, columnar epithelium; *c*, muscle layer. (90×)

rise to spermatids (*a*) which later cluster about the cells with strongly chromatophilic nuclei (*c*). These are probably sustentacular cells (*c*) which serve the spermatids in their metamorphosis to spermatozoa.

Although most cross sections of the testis show mature follicles, few reveal the actual openings of these follicles into the collecting tubule; yet on these sections alone depends the understanding of the rhythmical nature of follicular activity. Concurrently with the metamorphosing of spermatids to spermatozoa (Fig. 4*a*), the germinal epithelium, by mitotic division, again produces primary spermatocytes (*b*) which fill the follicles and expel the spermatozoa (*a*). The sustentacular cells, once numerous among the metamorphosing spermatids (*e*), are difficult to observe in the extruded follicular contents. It is quite evident, however, that the many groups of spermatozoa (*d*) seen descending the collecting tubule (*c*) are each the entire extruded content of one single follicle and not, as previously reported for *Panulirus penicillatus* (Matthews, 1951) the result of the activity of sustentacular cells. These groups of spermatozoa are further separated by a secretion (*f*) from the epithelial lining (*g*) of the tubule. As previously mentioned, this secretion mixes freely between the groups of spermatozoa.

Cross sections of the minute, proximal portion of the vas deferens (Fig. 5) reveal that the sperm mass (*a*) has not yet acquired its sheath. However, the columnar epithelial cells (*b*) are extremely active, and their secretions may be identical with this structure (Fig. 6*b*) seen in more distal sections.

Less than one third of the vas deferens is traversed by the descending sperm mass (Fig. 5*a*) before it acquires its spermatophoric sheath (Fig. 6*b*). This sheath is the product of the secretion from epithelial cells (*c*) which, although shorter than those previously encountered, show little specialized arrangement. There are no deep folds or crypts (*vide* Matthews, 1951: 363, fig. 7). The epithelial cells bounding the lumen are arranged in a single cell layer, and their secretion envelopes the sperm mass forming the spermatophoric sheath.

Cross sections through the distal portion

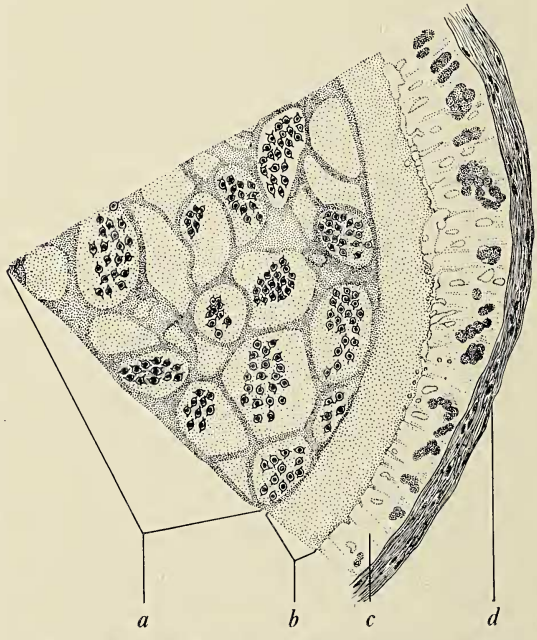

FIG. 6. Camera lucida drawing of a portion of a cross section of the vas deferens (distad to the portion illustrated in Fig. 5) showing: *a*, sperm mass; *b*, spermatophoric sheath; *c*, epithelial cells; *d*, muscle layer. (80×)

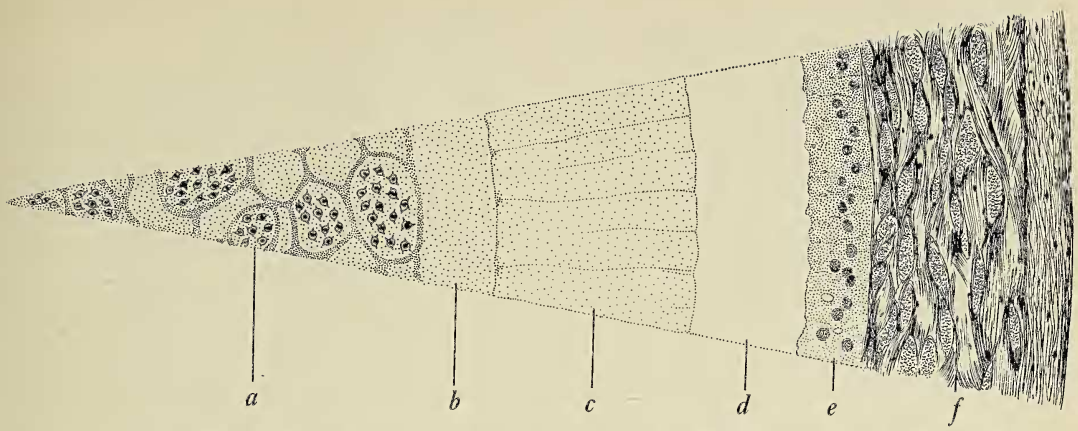

FIG. 7. Camera lucida drawing of a portion of a cross section of the distal region of the vas deferens showing: *a*, sperm mass; *b*, spermatophoric sheath; *c*, homogeneous matrix; *d*, space; *e*, epithelial cells; *f*, muscle layer. (90×)

of the vas deferens reveal what appears to be a sperm mass (Fig. 7*a*) surrounded by an extremely thick spermatophoric sheath (*b*, *c*). The reason for this misinterpretation lies in the fact that the sheath-forming secretion has the same consistency and staining properties as the matrix (Fig. 7*c*); both have an affinity for the cytoplasmic stain. Careful examination of the cross sections through this region, however, reveals a distinct diastem separating spermatophoric sheath (*b*) from matrix (*c*). Proof of the individual identity of these two secretions is further attested by placing the contents of the dissected, distal vas deferens in 0.1 N KOH. The outer matrix is readily soluble and soon disappears, whereas the inner spermatophoric wall, being less soluble, remains to disclose a highly coiled, continuous spermatophore.

The increased thickness of the muscular coat (Fig. 7*f*) probably plays a part in the expulsion of the spermatophoric mass during copulation; but whether it serves to segment the otherwise continuous spermatophoric tube into ampules is conjectural, since females with naturally occurring spermatophoric masses were not available for study. It seems very likely, however, that in either case the spermatozoa, encased in a spermatophoric sheath and enveloped in a hardened matrix, would require a mechanical method for their liberation.

Further indirect evidence in support of external fertilization is obtained on examination of the female, for although a large seminal receptacle is present, it is found not to connect internally with the female reproductive system, and probably serves as a receptacle on which to receive the spermatophoric mass of the male.

Because this mass becomes hard in a few hours when experimentally placed on glass plates immersed in sea water, it appears reasonable to assume that in naturally formed spermatophoric masses of *E. occidentalis* a similar change in consistency would necessitate mechanical liberation of the spermatozoa.

SUMMARY

The male reproductive system of the homarid-like, nephropsid lobster *Enoplometopus occidentalis* (Randall) is dissected and figured.

Cross sections throughout the testis reveal follicles in various degrees of maturity. An immature follicle, by mitotic division of the cells of its germinal epithelium, becomes filled with primary spermatocytes. These cells, by both heterotypic and homeotypic divisions, form spermatids.

Concurrent with the formation of primary

spermatocytes and spermatids, certain cells containing strongly chromatophoric nuclei arise from the germinal epithelium and become widely dispersed throughout the follicle. These sustentacular cells probably aid the spermatids in their metamorphoses into spermatozoa.

Each group of spermatozoa seen in the collecting tubule is the extruded contents of a single follicle. This extrusion is caused by the production of a new group of primary spermatocytes filling the follicle. A secretion from the epithelial cells of the tubule surrounds each group of spermatozoa. As this material traverses the proximal portion of the vas deferens, a secretion from the undifferentiated epithelial cells surrounds the sperm mass and forms the spermatophoric sheath.

No typhlosole-like structure is present in the enlarged vas deferens, but epithelial cells secrete a matrix which envelops the spermatophoric sheath. That spermatophoric wall and matrix are distinct secretions is attested both by the diastem which separates them and by the greater solubility of the matrix in 0.1 N KOH.

Although neither naturally occurring spermatophores nor the process of fertilization was actually observed, the facts that the matrix hardens and that the seminal receptacle does not open internally strongly suggest mechanical liberation of the spermatozoa and subsequent external fertilization.

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